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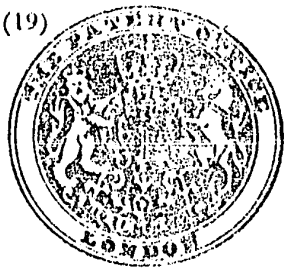

(54) PROCESS FOR THE CONVERSION OF STEROLS CONTAINED IN VEGETABLE AND ANIMAL OILS AND FATS INTO THEIR FATTY ACID ESTERS

(71) We, HARBURGER OEL-WERKE BRINCKMAN & MERGELL, a German kommanditgesellschalt, of Seehafenstrasse 2,21 Hamburg 90/West Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for the conversion of sterols with free hydroxyl groups, so-called free sterols, contained in vegetable and animal oils and fats, into their fatty acid esters by trans-esterification. The term "fatty acid", as used herein, means a carboxylic acid containing 2 to 26 carbon atoms.

Vegetable and animal oils and fats contain, as natural ingredients, sterols which are partly present in a chemically bound form as fatty acid esters and partly present in the free form, that is to say with free hydroxyl groups. Depending on their origin, these are either phytosterols or zoosterols. The most important representative of the latter group is cholesterol. Widespread phytosterols are sitosterols, stigmasterols, brassicasterol and campesterol. A further group consists of the mycosterols, which occur especially in fungi, yeasts and primitive plants; ergosterol is the most important example. Very recently, cholesterol has also been found in various vegetable fats and oils so that the classification mentioned above is somewhat artificial. Some data on typical sterol contents of the most important oils and fats are given below:

It is known that in processing edible oils and fats their sterols can undergo an undesired change, and that this above all affects the free sterols whilst changes in the sterol fatty acid esters occur only to a very slight extent, if at all. In the course of the bleaching which is usually carried out in the course of refining crude fats, activated fuller's earths, *inter alia*, cause dehydration of free sterols and hence the formation of secondary products.

For example, in this way, cholesterol yields di - (cholesten - (5) - yl - (3β)) - ether (dicholesteryl ether) and 3,5-cholestadiene. β-Sitosterol, stigmasterol and brassicasterol also form the corresponding diethers and hydrocarbons. In addition, some polar and non-polar secondary compounds, which have not yet been identified are also produced. During hardening of fat, the hydrogenation reaction can also lead to irreversible side-reactions of free sterols and hence to the production of undesired secondary products, such as cholestanol (dihydrocholesterol), coprostanone, coprostanol and epicholestanol.

In this context it has already be proposed to esterify the free sterols contained in oils and fats, prior to hardening, with known esterifying agents such as organic monocarboxylic acids, monocarboxylic acid anhydrides or monocarboxylic acid chlorides (see German Offenlegungschrift No. 1,617,035). The adverse working conditions of this method namely high temperatures, long reaction times and the corrosive properties of the esterifying agents or esterifying catalysts, militates against the use of this method in practice.

It has been known for a long time that phytosterol and its esters display a strong hypocholesterolaemic action, that is to say a lowering of the level of cholesterol in the blood or an inhibition of the formation of cholesterol in the blood, if they are added to vegetable oils. However, a prerequisite for this effect is a higher content of such sterols than is usually to be encountered in natural oils and fats. But at sterol contents exceeding about 0.5% oils do not remain bright at room temperature but are cloudy, since these sterols are rather sparingly soluble in oil. This decisively diminishes the usefulness of such oils, for example as salad oils, for the manufacture of for example, mayonnaise. It has therefore been proposed to employ for this purpose, instead of the free phytosterols, their fatty acid esters, which are readily soluble in oils at room tem-

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perature and which therefore make it possible for the oils to contain 0.5 to 10% by weight of sterols in the form of their esters (see, for example, German Offenlegungsschrift No. 2,035,069). According to this proposal, the requisite sterol esters are manufactured from free sterols by esterification with organic monocarboxylic acids after the free sterols have been isolated from oils or fats or other natural sources. Of course, this method is rather involved and expensive.

The purpose of the present invention is to protect free sterols contained in vegetable and animal oils and fats against possible changes during processing in a simple and effective manner and equally to convert higher proportions of added free sterols into a readily soluble form. It has been found that both requirements can be met by means of a modified form of the trans-esterification process.

According to the present invention there is provided a process for the conversion of free sterols, contained in vegetable and animal oils and fats, into their corresponding fatty acid esters by trans-esterification in a homogeneous phase and at elevated temperature, in the presence of alkali metal alcoholates or alkali metals as catalysts, which is characterised in that vegetable and/or animal oils and fats containing sterols are mixed with 1.0 to 1.1 equivalents, relative to their free sterol content of fatty acid esters of monohydric aliphatic alcohols with 1 to 4 carbon atoms and the mixture is subjected to trans-esterification conditions, with simultaneous removal of the monohydric alcohols liberated. It is to be understood that the fatty acid esters of the monohydric aliphatic alcohols can be derived from acids other than those present in the oil or fat; as indicated above, they contain 2 to 26 carbon atoms in the ester moiety.

The oils and fats treated according to the present invention can be used for the manufacture of hardened (hydrogenated) fats. The vegetable oils treated according to the invention can also be used as salad oils, for the manufacture of mayonnaise and as a constituent of mixtures of fats for the manufacture of margarine.

By the process according to the invention, free sterols contained in vegetable and animal oils and fats are substantially quantitatively converted into their fatty acid esters, independently of the actual free sterol content, so that added free sterols are also included in the reaction. In contrast, in the customary trans-esterification of edible fats which, as is known, is carried out without additives, apart from catalysts, free sterols are only partially converted into their fatty acid esters. A particular advantage of the process of this invention, which produces above all higher contents of free sterols, is that the products of the processes are practically free of partial glycerides, especially diglycerides, which are produced in an amount equivalent to the free sterol content if fatty acid monoesters are not used, that is to say in the customary trans-esterification of edible fats; oils and fats containing partial glycerides are known to have disadvantageous properties for various end uses.

The fatty acid monoesters required for the process according to the invention can readily be obtained by alcoholysis of oils and fats or by esterification of fatty acids with methanol, ethanol, propanols or butanols. If the monoesters corresponding to the oil or fat to be treated (esters of their total fatty acids) are used, the fatty acid spectra of the particular starting material and of the product of the process remain the same. Of course, monoesters of individual fatty acids or fatty acid mixtures of different composition can also be used. In the process according to the invention, 1 mol of free sterol or sterol mixture is equivalent to 1 mol of fatty acid monoester or fatty acid monoester mixture, from which the proportions of fatty acid monoester to be added can easily be calculated.

The crude oils and fats to be treated according to the invention are generally first freed of mucins, de-acidified and dried, in the usual manner; after carrying out the process according to the invention, the product may then be copiously washed with water to remove the catalyst, treated with small amounts (for example 0.5% by weight) of activated fuller's earth and subsequently deodorised. Excess, unreacted fatty acid monoester is generally removed by the deodorising process. In oils and fats which are subsequently to be hydrogenated, the bleaching and deodorising can be carried out after hardening.

The process according to the present invention is suitably carried out in closed, stirred vessels which can be evacuated and which are provided with heating and cooling devices and the customary fittings, including a connection for introducing nitrogen. After introducing the oil or fat which is to be processed, and the fatty acid monoester, optionally after addition of free sterol, the catalyst, which should be finally distributed (generally about 0.2% by weight of alkali metal alcoholate or 0.02% by weight of alkali metal), is introduced with the stirrer running and thereafter the mixture is heated to 90—160°C, preferably to 120—125°C, whilst evacuating it down to a final vacuum of less than 20 mm Hg. At the same time, a slow stream of nitrogen is introduced until the process is complete in order to accelerate the removal of the monohydric alcohol liberated and hence to accelerate the reaction in the desired direction. This is usually reached after 1 to 2 hours in the case of natural oils and fats. Greater amounts of added sterol require longer reaction times, up to a general maximum of 6 hours. The process can also be carried out continuously or semi-continuously.

The free sterols are precipitated from their solutions as digitonides by means of digitonin, but the slightest changes in structure annul this precipitatability; the quantitative determination of the total sterol and of the free sterol, from the difference between which the chemically bound sterol can be calculated, can be used for analytical control of the process and of its products. In order to determine the total sterol content the fat in question first should be saponified, and the unsaponified matter extracted from the soap solution by means of ether or petroleum ether. The sterols, which are now all present in the free form, are then precipitated from the solution of the unsaponifiable matter, thus obtained, by means of digitonin (suitably as a solution), and are separated off and weighed. To determine the free sterol content an aliquot portion of the fat is dissolved without saponification and a digitonin solution is added. Only the free sterols contained in the fat sample are precipitated in this way and these are then isolated as described above. The difference in the two determinations gives the amount of chemically bound sterols (sterols in the ester form) in the fat. This procedure is based on the method of M. Klostermann and H. Opitz, Handbuch der Lebensmittelchemie (Foodstuffs Chemistry Handbook), Berlin 1969, Springer Verlag, Volume IV: Fats and Lipoids, page 781. Of course, thin layer chromatography methods can also be used; this also allows the detection of possible decomposition products of sterols which can form during the processing described. The partial glycerides can be tested by the DGF standard method C-V17 (Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart), and from this any diglyceride content can be calculated.

The process according to the invention is in principle applicable to all oils and fats which can be processed industrially; it ensures reliable protection against changes in the sterols, both due to bleaching and hardening in the course of processing, and during use. In this context, autoxidative changes, to which free sterols are known to be more prone than their esters, should be mentioned particularly.

Fats which have been pretreated according to the processes of this invention, bleached and hardened can be used as cooking fats, frying fats and baking fats (shortenings) and for the manufacture of margarine. An enrichment with free sterols, and their subsequent conversion into fatty acid esters, is particularly useful for vegetable oils, such as soya oil, sunflower oil, maize oil and rape oil. With contents of up to 10% by weight of sterols, in the form of their esters, these oils [illegible] appearance at room temperature. They can be used as salad oils and oils for frying chips, for the manufacture of mayonnaise and as constituents of margarine fat mixtures, for example. Their advantages are attributable to the stability of the sterol esters contained in them and therefore relate primarily to their nutritional-physiological properties.

The following Examples further illustrate the present invention.

EXAMPLE 1.

1,000 g portions of (Peruvian) fish oil, soya oil or sunflower oil, which had been freed of slime, de-acidified and dried, were heated with approximately 1.05 times the stoichiometric amount of the methyl ester of their total fatty acids, calculated relative to the content of free sterols, and with 0.2% by weight of sodium methylate (in the form of a powder), to 120°C under a vacuum of 5 mm Hg while stirring. The mixture was kept for 2 hours at this temperature whilst passing a slow stream of [illegible] nitrogen through it. It was then cooled to 90°C, washed three times with 1/4 l portions of distilled water to remove the catalyst, dried *in vacuo* and treated with 0.5% by weight of activated fuller's earth for 20 minutes. After filtering off the fuller's earth, the product was deodorised for 4 hours *in vacuo* at 5 mm Hg and 200°C in the usual manner.

The products of the process were then hydrogenated at 180°C in autoclaves, using a nickel-kieselguhr catalyst in an amount of 0.2% by weight of nickel based on the weight of oil until products of melting point 40°C were obtained.

For comparison purposes, identical amounts of the same oils which had been freed of slime, de-acidified and dried were bleached, deodorised and hydrogenated, in the same manner.

The amounts of fatty acid methyl esters added and the sterol contents are listed in the Table below, in which the symbols denote:

a) oil which has been freed of slime, de-acidified and dried (starting material);

b) oil which has been trans-esterified (treated according to the invention) with fatty acid methyl esters, bleached and deodorised;

c) oil which has been treated as under b) and hydrogenated;

d) bleached, deodorised oil;

e) hydrogenated oil.

	mg of sterols in 100 g. total	free	chemically bound
Lard	75–125	75–125	0–1
Suet	75	72	3
Peruvian fish oil	340	170	170
Cod liver oil	515	272	243
Coconut oil	80	63	17
Soya oil	320	210	110
Sunflower oil	330	200	130
Groundnut oil	250	195	55
Rape oil	345	50	295

TABLE

Type of oil	Fatty acid ester in % by weight	Type of treatment	mg of sterols in 100 g total	free	chemically bound
Fish oil	–	a)	345	75	170
	0.15	b)	342	2	340
	0.15	c)	340	2	338
	–	d)	132	12	120
	–	e)	250	88	162
Soya bil	–	a)	325	205	120
	0.20	b)	321	1	320
	0.20	c)	309	1	310
	–	d)	160	45	115
	–	e)	233	118	115
Sunflower oil	–	a)	325	195	130
	0.20	b)	320	1	320
	0.20	c)	305	1	304
	–	d)	220	102	118
	–	e)	248	116	122

The hydroxyl numbers of all the products had a value of around 1, which corresponds to a content of about 1% by weight of diglycerides.

Accordingly, the treatment according to the invention, of the oils has resulted in their free sterols being converted almost completely into the corresponding esters. The experiments also show that the contents of chemically bound sterols (sterol esters) are only reduced slightly by bleaching and hydrogenation and hence no structural change whatever has taken place. In contrast, the contents, especially of free sterols, of the oils which have not been so treated have decreased substantially during further processing, which is presumably largely attributable to structural changes.

EXAMPLE 2.

50 g of β-sitosterol (pure, commercially available product) and 42 g of sunflower oil fatty acid ethyl ester, corresponding to 1.1 times the calculated stoichiometric amount, were dissolved, with warming, in 1,000 g of sunflower oil which had been freed of slime, de-acidified, dried and freed of wax. The mixture was then trans-esterified under the conditions of Example 1, extending the reaction time to 6 hours. After eluting the catalyst, drying and deodorising, a light yellow oil was obtained, which after winterising, that is to say after removing the triglycerides which separated out at 5 to 7°C and which were practically free of sterol esters, remained bright on storage in a refrigerator. 100 g of the oil contained 4.84 g of total sterols and 4.82 g of chemically bound sterols in the form of their esters, corresponding almost precisely to the theoretically calculated amount. The hydroxyl number of the oil was 0.8, corresponding to a calculated 0.7% by weight of diglycerides.

A mixture of soya oil with 9.8% by weight of cholesterol and 8.0% by weight of soya oil fatty acid ethyl ester was treated in the same manner. The oil contained 10.1% by weight of cholesterol bound as the ester, was practically free of diglycerides and after winterising remained bright on storage in a refrigerator.

WHAT WE CLAIM IS:—

1. Process for the conversion of free sterols contained in a vegetable or animal oil or fat, into their corresponding fatty acid esters which comprises adding to the vegetable or animal oil or fat 1.0 to 1.1 equivalents, based on their free sterol content, of a fatty acid ester of a monohydric aliphatic alcohol with 1 to 4 carbon atoms and transesterifying the mixture at elevated temperature in the presence of an alkali metal alcoholate or alkali metal as catalyst, with simultaneous removal of the monohydric alcohol liberated.

2. Process according to claim 1 in which free sterol is added to the vegetable or animal oil or fat before the transesterification.

3. Process according to claim 1 or 2 in which the fatty acid ester added is derived from the same fatty acid as present in the vegetable or animal oil or fat.

4. Process according to any one of claims 1 to 3 in which the transesterification is carried out at a temperature from 90° to 160°C., reducing the pressure to below 20 mm./Hg.

5. Process according to any one of the preceding claims in which the vegetable or animal oil or fat is fish oil, soya oil or sunflower oil.

6. Process according to any one of the preceding claims in which the catalyst is sodium methylate.

7. Process according to claim 1 substantially as hereinbefore described.

8. Process according to claim 1 substantially as described in Example 1 or 2.

9. A vegetable or animal oil or fat in which the free sterol present has been converted into their fatty acid esters by a process as claimed in any one of the preceding claims.

10. Process for making a hardened fat which comprises hydrogenating a vegetable or animal oil or fat as claimed in claim 9.

11. Process for making margarine which comprises incorporating with the mixtures of fats a vegetable oil as claimed in claim 9.

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